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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	**Confirmation No. 7556**
Shirou SAWA et al.	:	Attorney Docket No. 2006_0177A
Serial No. 10/568,418	:	Group Art Unit 1612
Filed April 26, 2006	:	Examiner Gigi G. Huang
AQUEOUS INTRAOCULAR PENETRATION-PROMOTING EYE DROP	:	**Mail Stop: Amendment**

SUBMISSION OF RULE 132 DECLARATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

THE COMMISSIONER IS AUTHORIZED TO CHARGE ANY DEFICIENCY IN THE FEES FOR THIS PAPER TO DEPOSIT ACCOUNT NO. 23-0975

Sir:

Submitted herewith is a Rule 132 Declaration executed by the inventor, Mr. Shirou Sawa.

The Declaration sets forth the Experimental Examples 1 and 2 of the specification as discussed in the Applicants' last response dated July 7, 2008.

In view of the foregoing, it is respectfully submitted that the Rule 132 Declaration demonstrates the unexpected excellent effect of the claimed method.

Accordingly, it is respectfully submitted that the claimed method is not obvious from the teachings of the cited references, and allowance is solicited.

Respectfully submitted,

Shirou SAWA et al.

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In re Application of Confirmation No.: 7556

Serial No. 10/568,418 Art Unit: 1612

Shirou Sawa Examiner: Huang, Gigi Georgiana

Filed: April 26, 2006

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks

Sir,

I, Shirou Sawa declare that:

I was born in Tokushima Prefecture, Japan, in 1965;

I am a citizen of Japan and a resident of Kobe-shi, Hyogo Prefecture, Japan;

I graduated from Department of Chemical Engineering, Faculty of Engineering, The University of Tokushima, Tokushima, Japan in 1988;

I received the Master degree on the study of the chemical engineering at The University of Tokushima in 1990

I have been an employee of Senju Pharmaceutical Co. Ltd. Japan, since 1990 up to this time;

I have been engaged in research on formulation of eye drops;

I am one of inventors of the above-identified patent application;

The experiments set out below were conducted under my supervision and direction.

EXPERIMENT

Experimental Example 1

Penetration Test into the Aqueous Humor

A penetration test of sodium 2-amino-3-(4-bromobenzoyl)phenylacetate of the following formulations (Table 1) into the aqueous humor was carried out using trometamol and aminoethylsulfonic acid.

1. Test Material

The eye drops of Formulations 1 to 3 in Table 1 were prepared and used.

Table 1

Component	Formulation 1	Formulation 2	Formulation 3
Sodium 2-amino-3-(4-bromobenzoyl)phenyl acetate 3/2 hydrate	0.1 g	0.1 g	0.1 g
Boric acid	1.8 g	-	-
Trometamol	-	0.5 g	-
Aminoethylsulfonic acid	-	-	0.2 g
Concentrated glycerin	-	2.0 g	2.4 g
Sodium hydroxide	q.s.	-	q.s.
Hydrochloric acid	-	q.s.	-
Purified water	q.s.	q.s.	q.s.
Total amount	100 mL	100 mL	100 mL
pH	7.8	7.8	7.8

2. Test Method

Rabbits (KITAYAMA LABES Co., Ltd) which have no abnormal cornea were selected (n=5), and 50 μL of each test material (eye drops of Formulations 1 to 3) was administered once to the rabbits by using a pipette. The rabbits were euthanized by overdosing a solution of pentbarbital sodium 2 hours after the intraocular administration. After the external segment of the eye was washed with physiological saline, the aqueous humor was collected by using a syringe with a 27G injection needle. 160 μL of the collected aqueous humor was mixed with 160 μL of a mobile phase for pretreatment/ concentration as mentioned below,

and then the mixture was filtered with a membrane filter (0.45 μm). The filtrate was served as a sample of HPLC measurement and then the concentration of 2-amino-3-(4-bromobenzoyl)phenylacetic acid was determined under the HPLC condition mentioned below by using High Performance Liquid Chromatograph (Shiseido Co., Ltd., type: Nanospace SI-1).

<HPLC condition>

Detector: Ultraviolet spectrophotometer (wave length for measurement:266 nm)

Column: (for pretreatment) Capcell pak MF Ph, 4.0 × 20 mm (Shiseido Co., Ltd.)

Column: (for concentration) Capcell pak C18 MG S5mm 1.5 × 35 mm (Shiseido Co., Ltd.)

Column: (for analysis) Capcell pak C18 MG S5mm 1.5 × 250 mm (Shiseido Co., Ltd.)

Column temperature: a constant temperature around 40°C, room temperature only for concentration column

Mobile phase: (for pretreatment and concentration) phosphate buffer (pH 7.3)*: acetonitrile = 90:10 (v/v)

Mobile phase: (for analysis) phosphate buffer (pH 7.3)*: acetonitrile = 60:35 (v/v)

Injection amount: 70 μL × 2 = 140 μL

* Phosphate buffer (pH 7.3): 50 mM diammonium hydrogenphosphate buffer (pH 7.3) containing 5 mM tetrabutylammonium chloride

Table 2

Pump and valve switching schedule

Pump for analysis		Pump for pretreatment and concentration		Valve
Time(min)	Flow rate (mL/min)	Time (min)	Flow rate (mL/min)	Valve position
0.0	100	0.0	500	A
↓	↓	0.5	250	B
↓	↓	5.5	10	A
↓	↓	29.5	500	A
30.0	100	30.0	500	A

3. Results

With respect to the eye drop of Formulation 1 (no addition of an organic amine), the concentration of 2-amino-3-(4-bromobenzoyl)phenylacetic acid in the aqueous humor was 214 ± 46 (ng/mL) 2 hours after the intraocular administration. On the other hand, with respect to the formulation containing trometamol (the eye drop of Formulation 2) and the formulation containing aminoethylsufonic acid (the eye drop of Formulation 3), concentrations of 2-amino-3-(4-bromobenzoyl)phenylacetic acid in the aqueous humor were respectively 260 ± 45 (ng/mL) and 350 ± 123 (ng/mL) 2 hours after the intraocular administration (Table 3). The concentration of 2-amino-3-(4-bromobenzoyl)phenylacetic acid in the aqueous humor 2 hours after the intraocular administration increased about 1.2 times in case of the formulation containing trometamol and about 1.6 times in case of the formulation containing aminoethylsufonic acid compared to the eye drop of Formulation

1.

Table 3

	2-Amino-3-(4-bromobenzoyl)phenylacetic acid (ng/mL)
Formulation 1	214 ± 46
Formulation 2	260 ± 45
Formulation 3	350 ± 123

As can be seen above, the penetration of 2-amino-3-(4-bromobenzoyl)phenylacetic acid into the aqueous humor was elucidated to significantly increase by the addition of trometamol and aminoethylsulfonic acid which are an organic amine.

Experimental Example 2

Medicinal Efficacy Test in a Model Rabbit of Anterior Chamber Puncture

1. Test Animal

Male Dutch rabbits (Biotech Co., Ltd) with a body weight of about 2 kg was bred and acclimatized under the condition that the temperature was 23±2°C and the humidity was 55±10%. On the day of the test, the flare value of anterior chamber in the rabbits was determined by a laser flare cell meter (FC-1000, Kowa Company, Ltd.). The rabbits having a flare value of not more than 30 and no abnormality in general condition were selected and used for the test.

2. Test Material

The eye drops of Formulations 4 to 6 in Table 4 were prepared and used.

Table 4

Component	Formulation 4	Formulation 5	Formulation 6
Sodium 2-amino-3-(4-bromobenzoyl)phenylacetate 3/2 hydrate	0.1 g	0.1 g	0.1 g
Boric acid	1.1 g	-	-
Borax	1.1 g	-	-
Benzalkonium chloride	0.005 g	-	-
Polysorbate 80	0.15 g	-	-
Povidone (K-30)	2 g	-	-
Disodium edetate	0.02 g	-	-
Sodium sulfite	0.2 g	-	-
Sodium dihydrogenphosphate dihydrate	-	-	0.05 g
Aminoethylsulfonic acid	-	0.5 g	1.0 g
Concentrated glycerin	-	2.2 g	2.6 g
Sodium hydroxide	q.s.	q.s.	q.s.
Purified water	q.s.	q.s.	q.s.
Total amount	100 mL	100 mL	100 mL
pH	8.3	7.8	7.0

3. Test Method

At 30 minutes before the puncture of the anterior chamber, 1,000 U/kg of heparin (Heparin sodium injection, Ajinomoto Co., Inc.) was administered into the rabbit's ear vein. A disposable intraocular injection needle (30G×3/4, Nipro Medical Industries, Ltd.) was curvedly punctured from the corneal center side at

the position which is about 1 mm apart from the 1 o'clock position of rabbit's eyeball limbus to collect 80 μL of the anterior aqueous humor via the cornea (puncture of anterior chamber). The flare value (photon count/msec) in the anterior chamber was determined 30 minutes after the puncture of anterior chamber using a laser flare cell meter (FC-1000, Kowa Company, Ltd.). 50 μL of the test material was administered 24 hours before the puncture of anterior chamber. Meanwhile, nothing was administered to the control group.

An inhibition rate of inflammation after the puncture of anterior chamber was calculated according to the following equation.

Inhibition rate (%) = ((Average flare value in the anterior chamber of the control group) minus (Average flare value in the anterior chamber of the test material administered group))/ (Average flare value in the anterior chamber of the control group) × 100

4. Results

Table 5 shows the inhibition rate of inflammation after the puncture of anterior chamber, which was calculated by the measured flare value in the anterior chamber. The inhibition rate of the formulation in which no aminosulfonic acid was added (Formulation 4) was 0.3% at 24 hours after the puncture. On the other hand, the inhibition rate of Formulation 5 was 25.5% and the inhibition rate of Formulation 6 was 73.9%, both of the formulations being combined with aminoethylsulfonic acid.

Table 5

	Inhibition rate (%)
Formulation 4	0.3 (n=6)
Formulation 5	25.5 (n=7)
Formulation 6	73.9 (n=10)

It is declared by the undersigned that all statements made herein of undersigned's own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 24 day of July, 2008

Shirou Sawa

Shirou Sawa